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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

ATTY.'S DOCKET: KATO=15

In re Application of:	Art Unit: 1761
Yukihisa KATO	Examiner: Curtis SHERRER
Appln. No.: 09/144,851	Washington, D.C.
Date Filed: September 1, 1998	Confirmation No. 5275
For: FRUIT VINEGAR FROM RAW MATERIAL FLAVORFUL ACID CITRUS...	April 14, 2004

REPLY BRIEF ON BEHALF OF APPELLANTS

Honorable Commissioner for Patents
U.S. Patent and Trademark Office
Mail Stop Appeal Brief-Patents
2011 South Clark Place
Crystal Plaza Two, Lobby, Room 1B03
Arlington, Virginia 22202

Sir:

Submitted herewith is Applicant's Reply Brief in triplicate.

RESPONSE TO EXAMINER'S ARGUMENT

The Examiner has cited *In re Levin*, 84 USPQ 232 (C.C.P.A. 1949) in support of a finding of obviousness of the presently claimed invention. In finding a recipe for a dairy product obvious, the Court stated, "The difficulty with appellant's argument is that no specific coopération between his selected ingredients has been shown as required by the rule laid down in the last two cases of this court hereinafter

cited." *In re Levin*, 84 USPQ 232 (C.C.P.A. 1949), 234. The Examiner's position is that, despite the reference cited above not disclosing the amounts of the fruit juice used, the specifically claimed citric acid content, it is considered that in view of the court's holding in *In re Levin*, it would have been obvious to those in the vinegar processing industry to modify these parameters as they are result effective variables that are commonly optimized.

First of all, *Levin* related to a formulation for a food product. In the present case, however, the removal of citric acid was effected in order to promote a fermentation reaction, as acetic acid bacteria would not convert the sugars and alcohol to acetic acid in the presence of excess citric acid. This is not at all the same as eliminating one ingredient in a recipe, as the removal of excess citric acid in the present invention directly affects the feasibility of the fermentation process.

Second, it is well known in the fermentation art that addition of an alkaline agent to fruit juice severely affects the flavor of the resulting fruit vinegar (specification, page 4, lines 8-16). Seike first subjects the fruit juice to enzymatic treatment with pectinase to hydrolyze

the pulp in the juice, and then adds sodium citrate to the juice to adjust the pH to 4.6. There is neither disclosure nor suggestion of eliminating the citrate ion—only of raising the pH with an alkaline salt of citric acid.

When Seike adds sodium citrate to the fruit juice, the pH is raised because Seike has added an alkaline agent. An alkaline agent is known to severely affect the flavor of the resulting fruit vinegar. However, in the present case, calcium carbonate is added, which **precipitates** calcium citrate. In other words, the alkaline agent, calcium carbonate, which converts citric acid to insoluble calcium citrate, is not present as an ionized species in the fruit juice, but rather precipitates the citrate ion and thus is effectively removed from the fruit juice.

An English translation of Seike was filed April 4, 2002. As can be seen from the description of lines 17-24 on page 6 and "Brief Explanation of the Drawing" on page 16 and Figure 1 on page 17 of the translation, it is apparent that acid adjustment in Seike means raising the pH of citrus juice to 4.6 by adding sodium citrate. However, as noted in the present specification, sodium citrate, an alkaline agent, adversely affects the flavor of vinegar.

The Examiner alleges that, because Jackson provides those with ordinary skill in the art a teaching to reduce juice acidity with calcium carbonate, those of ordinary skill would perform such a step.

It is respectfully submitted that, although Jackson teaches deacidification of grape juice using calcium carbonate, and deacidification of wine using calcium carbonate, these teachings are limited to production of wine from grapes.

Jackson uses calcium carbonate to solve the problem that citric acid produces an undesirable flavor in wine. However, citric acid does not produce an undesirable flavor in vinegar. The deacidification, *i.e.*, reduction of citric acid in the juice, in the present invention is a means to effect acetic acid fermentation because the acetic acid bacteria cannot ferment at a pH that is below that at which they function. Jackson teaches nothing with respect to production of vinegar, which is a fermentation process which occurs after the production of wine, or ethanol.

Jackson states, "Although widely used, calcium carbonate has a number of disadvantages." page 279, right column, which would lead one skilled in the art away from using calcium carbonate. However, the use of calcium carbonate in the present invention is used to avoid the drawback in Seike that sodium citrate, an alkaline agent, adversely affects the flavor of vinegar, as the precipitation of calcium citrate in the present invention removes the alkaline agent from the juice solution.

The Examiner asserts on page 5, fourth paragraph, of the Examiner's Answer, "It is noted that on page 5 of the Brief, that appellants state that the present invention provides a method for preparing vinegar from citrus fruit juices that contain a higher percentage of citric acid than apple juice or grape juice…" This asserted aspect of the invention is not claimed nor disclosed and therefore is not found persuasive."

It is respectfully submitted that this aspect of the invention is indeed disclosed and claimed in the instant application. Claim 21, the independent claim on appeal, limits the juice which is fermented to a member of the group consisting of lemon juice, lime juice, yuzu juice, kabosu

juice, sudachi juice, and shii kuwasa juice. These juices are naturally flavorful acid citrus fruit juices that have a high citric acid content of 3-8%, as noted on page 5 of the specification. It is well known by those in the art that a citric acid content of 3-8% is much higher than that of apple juice and grape juice. This is shown in the references filed April 4, 2002, and "RSK-Values, the Complete Manual, 1987, English Translation", submitted herewith.

It should be noted from "The Complete Manual" that the amount of citric acid in apple juice is from 50-200 mg/liter and the amount of citric acid in grape juice is from 200-500 mg/liter. The amount of citric acid in lemon juice, however, is 100 to 210 **grams** per liter, a 1000-fold difference.

The specification as filed at page 5, lines 11-20, characterizes the present invention as a method for producing fruit vinegar using a flavorful citrus fruit juice with a citric acid content reduced to 50% by weight or less of the natural citric acid content (3 to 8%) in a flavorful acid citrus fruit juice by acid reduction treatment, as a means for producing fruit vinegar with natural flavor unique to flavorful acid citrus fruit. Thus, it is clear that the

present invention provides a method for preparing vinegar from citrus fruit juices that contain a higher percentage of citric acid than apple or grape juice.

As has been emphasized throughout the prosecution of this application, citric acid is removed from citrus fruit juice in order to improve the flavor of the vinegar so produced.

CONCLUSION

There is nothing in the combination of Seike and Jackson that would lead one skilled in the art to make vinegar from citrus fruit juice by removing at least some of the citric acid prior to fermentation. There is nothing in the combination of Seike, Jackson, and Castillon et al. that would lead one skilled in the art to make vinegar from citrus fruit juice by removing at least some of the citric acid prior to fermentation, fermenting the juice, and using an ultrafiltration membrane to clarify the resulting vinegar.

Claims 21-24 and 27-31 are patentable over the cited references because there is no motivation to combine the references to arrive at the herein claimed invention, namely,

that at least some citric acid is removed from citrus fruit juice prior to fermentation.

Wherefore, it is respectfully requested that this Board reverse the rejection of claims 21-24 and 27-31.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By Anne M. Kornbau
Registration No. 25,884

AMK:msp
Telephone No.: (202) 628-5197
Facsimile No.: (202) 737-3528
G:\BN\T\toda\Kato15\Pto\Kato15.replybrief.doc

R S K - Values
The Complete Manual

Guide Values and Ranges of Specific Reference Numbers for Fruit Juices and Nectars, Including the Revised Methods of Analysis

VdF
Verband der deutschen Fruchtsaftindustrie e.V.
Bonn

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Responsible for translation:
FLÜSSIGES OBST GmbH, Schönborn, W.-Germany
Translated by:
I. Schöberl, Qualified Translator, Schönborn, W.-Germany
Proof-read by:
Dr. A.G.H. Lea, Food Chemist, Reading, Great Britain
Dr. A. Rother, Food Chemist, Heidelberg, W.-Germany
J. Ellis, B.A. Chem., Katzenelnbogen, W.-Germany
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3. RSK-Values for Different Fruit Juices with Comment

3.1 Apple Juice

The production of cider apples with high acid contents is decreasing more and more which means that apple juice manufacturers are dependent on rejected dessert fruit. Because their content of titratable acids is too low, raw juices made from these "sweet" apples, i.e. low-acid juices frequently do not come up to trade usage regarding industrial fruit juice as is set down in the Leitsätze für Fruchtsäfte. In order to come up to trade practice, these juices must be blended with high-acid apple juices.

Comment

1. Relative Density 20°/20°C

Not all apple juices come up to the minimum value established as trade practice, i.e. 1.0450. Juices with low extract contents can be blended with juices showing higher extract contents. A juice with a high extract value, however, must not be adjusted to the minimum value of relative density as set down in the Leitsätze für Fruchtsäfte, by watering. If an apple juice concentrate is reconstituted to single strength, the organoleptic and analytic characteristics must be equivalent to those of the original juice (article 1 (5, 6) EC-Directive on Fruit Juices). The minimum value of relative density results from native fruit components.

2. Reduction free Extract

Reduction-free extract means the sum of "non-sugar substances" dissolved in the juice. Usually included are the content of fruit acids, mineral substances and D-sorbitol. Since juices made from low-acid apples have extremely low acid and D-sorbitol contents, the specified minimum value in some cases may not be reached. Approved handling operations have virtually no effect on the reduction free extract.

3. Monosaccharides, Disaccharides

The most important sugars found in apples are glucose, fructose and sucrose. The content of sucrose depends on the degree of maturity and can make up one third of the total sugar content, but it is usually lower. The fructose content is about 2-3-times that of glucose. In exceptional cases, the glucose-fructose ratio can be slightly lower than 0.30. In combination with other reference numbers, values higher than 0.50 indicate a treatment with high-glucose sugars.

Tab. 1: RSK-Values Apple Juice

		guide value		range from	range to	central value
A. sensory analysis						
colour/appearance	(points)	min.	3	2	4	3
aroma	(points)	min.	3	3	6	4
flavour	(points)	min.	5	5	10	7
B. chemical analysis						
relative density 20 °/20 °C*)		min.	1.0450	1.0450	1.0670	1.0488
Brix, ref. corr.*)		min.	11.18	11.18	14.01	12.08
soluble solids*)	g/l	min.	116.8	116.8	148.1	126.7
titratable acids (pH 7.0)*)						
expr. as tartaric acid	g/l	min.	5.0	5.0	8.5	6.6
expr. as mval/l		min.	66.7	66.7	113.3	86.7
ethanol*)	g/l	max.	3.0	—	—	—
volatile acids*)						
expr. as acetic acid	g/l	max.	0.4	—	—	—
sulphur dioxide, total*)	mg/l	max.	10	—	—	—
lactic acid*)	g/l	max.	0.5	—	—	—
D-malic acid	g/l		n.d.			
citric acid	mg/l		—	50	200	100
tartaric acid	g/l		n.d.			
glucose	g/l		—	18	35	26
fructose	g/l		—	55	80	65
glucose-fructose ratio		max.	0.5	0.3	0.5	0.40
sucrose	g/l		—	5.0	30.0	15.0
D-sorbitol	g/l	min.	2.5	2	7	4
reduction-free extract	g/l	min.	18	18	29	22
ash	g/l	min.	2.1	1,9	3.5	2.55
alkalinity number		min.	11	11	14	13
potassium (K)	mg/l	min.	1000	900	1500	1200
sodium (Na)	mg/l	max.	30	—	—	—
magnesium (Mg)	mg/l		—	40	70	52
calcium (Ca)	mg/l		—	30	120	59
chloride (Cl)	mg/l	max.	50	—	—	—
nitrate (NO_3)	mg/l	max.	10	—	—	—
phosphate (PO_4)	mg/l	min.	160	130	300	220
sulphate (SO_4)	mg/l	max.	150	—	—	—
formol number (ml 0.1 mol NaOH/100 ml)			—	2.5	10	4.5
proline	mg/l	max.	15	—	—	8

*) cf. chapter 2.1, n.d. = not detectable

4. Fruit Acids

The total acid content in apple juices depends mainly upon the amount of L-malic acid. The quantity of L-malic acid which can be determined enzymatically is higher than the numeric value of the titratable total acid expressed as tartaric acid. If the content of L-malic acid (enzymatic determination) is lower than the value for titratable total acid (expressed as tartaric acid, see 6.30), the juice has to be analysed for lactic acid and other fruit acids. Considering the limits of error of the different methods, the content of total malic acid (chemical determination) corresponds to the L-malic acid (enzymatic determination). Therefore, the addition of synthetic DL-malic acid can be recognized from the result of both analyses. The content of citric acid does not exceed 200 mg/l. Higher values indicate the addition of citric acid or other fruit juices, as e.g., pear juice. In exceptional cases, the minimum value for the range laid down for citric acid may not be reached. As a rule, the value for titratable acid of juices made of dessert apples and those from growing areas located in warmer climates is lower than the minimum value of the range. The upper value of the range, on the other hand, may be exceeded considerably by high-acid varieties.

5. Biogenic Acids (microbial origin) and Ethanol

Biogenic acids and ethanol are almost never found in apple juices. Excess values indicate the processing of rotten fruit or microbial changes during production and storage of the juice.

6. Mineral Substances

6.1 Ash

There is a correlation between the content of mineral substances and the reduction-free extract (Mineral substances make up approx. 10% of the reduction-free extract). The different mineral substances detected in the ash show only slight variations in their relations to each other. If the ash content of a juice is lower than 1.9 g/l, it may be supposed that the juice has been adulterated with water or that the concentrate has been diluted beyond the degree which is authorized. A treatment with appropriate bentonite has virtually no influence on the mineral composition of the juice.

6.2 Alkalinity Number

The alkalinity number expresses the alkalinity-ash ratio, and normally varies between 12 and 14. Values lower than 11 indicate inadmissible treatments. Authorized handling operations have almost no influence on the ash content, ash alkalinity and thus the alkalinity number.

6.3 Potassium

The potassium content varies within narrow limits; on average it amounts to 48% of the ash content.

6.4 Sodium

Normally, the sodium content is lower than 20 mg/l. Values higher than 30 mg/l may be due to special growth conditions, because fruit grown near the sea may have higher sodium contents. But higher contents may also be due to inadmissible treatments.

6.5 Calcium

The calcium content is seldom higher than 80 mg/l, yet it may be clearly increased if the water used for concentrate reconstitution has inappropriate characteristics (in this case, e.g. the magnesium and nitrate content may be accordingly increased).

6.6 Magnesium

The magnesium content varies within comparatively narrow limits. Quite often, the specified minimum value is fallen below by raw juices made of "sweet" apples. In some exceptional cases, it is not reached by "sour" apple juices.

6.7 Phosphate

A phosphate content which is quite high as compared to the ash content indicates the addition of phosphate-salts. If the phosphate content is lower than the specified minimum content, it may be supposed that the juice has been diluted.

6.8 Nitrate

Normally the nitrate content is lower than 5 mg/l. In combination with reduced contents of other values, higher contents indicate an adulteration with nitrate-containing water. If a concentrate is reconstituted with nitrate-containing water the contents of the other mineral substances are not reduced. If in concentrate reconstitution, nitrate contents of more than 10 mg/l occur, it may be supposed that the water used has inappropriate characteristics.

6.9 Chloride

In un-treated juices, the chloride content is normally lower than 15 mg/l. Higher chloride contents may be due to special growing conditions, if fruit grown near the sea has been processed. In this case, the chloride concentration does not correlate with the sodium content. Chloride values which are higher than the guide value, indicate inadmissible additions of salts or, in the case of concentrates, the use of water not having the appropriate characteristics for reconstitution.

7. Formol Number

The formol number varies widely. The specified minimum value may not be reached by raw juices made from "sweet" apples. Protein-containing fining agents have almost no influence on the formol number.

8. Proline

Apple juices have a low proline content. As a rule it is lower than 15 mg/l. Higher values indicate that other juices have been added.

9. D-Sorbitol

Apple juices always contain D-sorbitol. The D-sorbitol content varies within relatively wide limits. Juices made from "sweet" apples tend to lower values. In exceptional cases the D-sorbitol values are slightly lower than the specified minimum value.
In juices with high acid and high extract values the maximum value may be exceeded.

10. Hydroxymethylfurfural (HMF)

The HMF content is not a criterion by which the adulteration of a juice can be judged. In apple juices which have been properly manufactured, stored and filled, only traces of HMF occur. Even in properly manufactured concentrates HMF may be formed, the concentration being increased in the course of, even proper, filling. Values of approx. 20 mg/l or more together with a cooked or bready flavour, indicate excessive thermal stress; such stress, however, can be prevented by technological means.

3.2 Apricot Puree and Juice

In industry, mainly apricot puree is processed. The following values are therefore referred to kilograms. Application of these RSK-values to apricot juice is possible providing that the relative density value is taken into account. In comparison with apricot puree, the following mineral substances show a tendency towards the lower range limit: calcium, phosphate, sulphate and potassium.

Comment

1. Relative Density 20°/20°C

Values lower than 1.040 indicate that unripe fruits have been used.

9. Ascorbic Acid

The L-ascorbic acid content is dependent on the berry variety and the method of production used.

3.4 Grape Juice

For the production of grape juice, "vitis vinifera" vine types are used. According to the EEC order "über die gemeinsame Marktorganisation für Wein" *(EEC-order on a common market organisation for wine; see EEC 337/79) these vines (see EEC 2005/70) must have been authorized or recommended. According to this order, grape juice is the product obtained from fresh grapes, fermentable but unfermented, which has been treated in a way that it is suited for direct consumption, and which has a maximum alcohol content of 1% by vol. According to the EC-Directive on Fruit Juices, the following procedures may be used, too:*

1. Desulphurization with physical procedures

2. Partial deacidification.

Comment

1. Relative Density 20°/20°C

In the Federal Republic of Germany mainly grape juices imported from southern growing areas are marketed. Due to the special climatic growing conditions, the relative density of white grape juices is seldom lower than 1.068, whereas that of red grape juices is seldom lower than 1.070. A relative density value of more than 1.080 is possible and does not necessarily imply that concentrated grape juice has been used. Dilution of grape juices with high extract values is not permitted. A relative density of 1.065 is still in keeping with German trade usage. The relative density may be as low as 1.055 but only in juices from grapes grown in Germany or comparable growing areas.

2. Reduction free Extract

The reduction free extract value of grape juices may be lowered due to the crystallization of potassium tartrate. The reduction-free extract is reduced by 1.8 g/l for each g/l of tartaric acid crystallized. This fact has to be considered in juice evaluation. In exceptional cases, grape juices with high acid values may exceed the maximum range value. As compared to juices from white grapes, juices made from red grapes tend to higher values.

Grape Juice

3. Monosaccharides, Disaccharides

The glucose-fructose ratio is seldom higher than 1.0. Values below 0.9 may be due to slight fermentation. The central value of glucose is about 79 g/l, and that of fructose is about 81 g/l. Since they are not very meaningful, ranges for glucose and fructose were not set down. Using the chemical methods of analysis indicated, sucrose cannot be detected in grape juice except for freshly pressed juice. Sucrose values determined according to "Luff-Schoorl" are not taken into account if they are within the tolerable limits of error of the methods used.

Tab. 4: RSK-Values for Grape Juice

		guide value		range from	range to	central value
A. sensory analysis						
colour/appearance	(points)	min.	3	2	4	3
aroma	(points)	min.	3	3	6	4
flavour	(points)	min.	5	5	10	7
B. chemical analysis						
relative density 20 °/20 °C**)		min.	1.065	1.065	1.080	1.070
Brix, ref. corr.*)		min.	15.88	15.88	19.30	17.03
soluble solids*)	g/l	min.	169.1	169.1	208.4	182.2
titratable acids (pH 7.0)*)						
expr. as tartaric acid	g/l	min.	6.0	6.0	12	8
expr. as mval/l		min.	80.0	80.0	160	106.7
sulphur dioxide, total*)	mg/l	max.	10	—	—	—
ethanol***)	g/l	max.	3.0	—	—	—
volatile acids*)						
expr. as acetic acid	g/l	max.	0.4	—	—	—
lactic acid*)	g/l	max.	0.5	—	—	—
L-malic acid	g/l	min.	3	—	—	5
citric acid	mg/l	max.	500	200	500	300
tartaric acid	g/l	max.	—	—	—	3.5
free tartaric acid	g/l	max.	1	—	—	—
glucose-fructose ratio		max.	1.0	0.90	1.03	0.97
sucrose	g/l	s. comment				
reduction-free extract	g/l	min.	20	18	32	22
ash	g/l	min.	2.2	2.2	5	3
alkalinity number		min.	11	11	14	12
potassium (K)	mg/l	min.	950	900	2200	1250
sodium (Na)	mg/l	max.	30	—	—	—
magnesium (Mg)	mg/l	min.	80	75	150	95
calcium (Ca)	mg/l	max.	230	100	250	170

		guide value		range from	range to	central value
chloride (Cl)	mg/l	max.	50	—	—	—
nitrate (NO_3)	mg/l	max.	15	—	—	7
phosphate (PO_4)	mg/l	min.	300	300	550	380
sulphate (SO_4)*)	mg/l	max.	350	—	—	—
formol number (ml 0.1 mol NaOH/100 ml)		min.	11	10	30	15
proline	mg/l	min.	150	150	1000	—

*) cf. chapter 2.1
**) For products from German grapes and such of similar growing areas, the value of 1.055 is still tolerable as lower limit.
***) According to EC-Order No. 337/79 of February 2, 1979, concerning the Common Market Organization for Wine, Annex 2, No. 6, grape juice contains a maximum of 1% by vol. of ethanol.

4. Fruit Acids

The quantity of titratable acids in grape juice is mainly determined by the presence of L-malic acid and tartaric acid. The sum of the content of tartaric acid and L-malic acid is higher than the amount of titratable acids.

4.1 L-Malic Acid

The content of L-malic acid is largely dependent on the respective varieties and climatic conditions. Consequently, a specification of ranges is not very useful. The content of L-malic acid decreases with increasing fruit maturity, but only in exceptional cases, this value may go down to 2.5 g/l.

4.2 Tartaric Acid/"Free Tartaric Acid"

The tartaric acid content of grape juices is dependent on the degree of maturity, fruit variety and the handling operations used (i.e. de-acidification, possible crystallization of potassium tartrate). The addition of tartaric acid can be detected by calculating the "free tartaric acid" content. In this, the proportion of potassium to tartaric acid (39:150) as it is found in crystallized potassium tartrate is used as a basis. The arithmetical amount of "bound tartaric acid" is calculated as follows:

$$\frac{\text{potassium (g/l)} \times 150}{39} = \text{bound tartaric acid (g/l)}$$

The content of "unbound tartaric acid" = "free tartaric acid" is calculated from:

tartaric acid (g/l) — "bound tartaric acid" (g/l) = "free tartaric acid" (g/l).

Normally, the determinable content of tartaric acid is smaller than that calculated on the basis of the potassium content. If a juice has a low potassium content but a high proportion of tartaric acid, it may happen that the tartaric acid is not "bound" completely by the potassium contained in the juice. Natural concentrations of "free tartaric acid" are mainly found in grape juice made from Rabosa-grapes, a grape variety with high acid contents (High contents of tartaric acid may also be found in products made from unripe fruit).

The addition of tartaric acid is considered a proven fact if these exceptions (high malic acid values in unripe fruit) do not apply and if the "free tartaric acid" value is higher than 1.5 g/l. Even small amounts of tartaric acid have to be considered critically.

4.3 Citric Acid

In exceptional cases, juices with extremely high acid values (also unfermented juices from grapes showing noble rot which are, however, not used for the preparation of grape juice) can show values which are higher than 500 mg/l. Concentrations below the specified minimum value may indicate dilution through water addition.

5. Mineral Substances

5.1 Ash

In the case of ash contents below 2.2 g/l it may be supposed that an illegal dilution has been performed or that concentrates or semi-concentrates which are not suited for the production of grape juice have been processed. The loss of 1 g/l tartaric acid through potassium tartrate crystallization means that the ash content is reduced by about 0.46 g/l.

5.2 Alkalinity Number

The ash alkalinity-ash ratio can give substantial information as to the handling operations used. Alkalinity numbers lower than 11 may be caused by a higher proportion of "acid" ash components e.g. sulphate, phosphate, chloride. In de-acidified juices, an increased alkalinity number normally occurs together with higher calcium values and generally also with higher potassium values.

5.3 Potassium

Freshly pressed grape juices seldom contain potassium concentrations lower than 1 400 mg/l. Considerable reductions may be due to the natural formation of potassium tartrate (e.g. through a loss of 1 g/l tartaric acid, the potassium content is reduced by 260 mg/l). In the case of high calcium concentrations — which indicate a de-acidification treatment with calcium salts — the potassium content

will be correspondingly higher; the latter can be explained by the lower rate of potassium tartrate crystallization.

Normally, the potassium content makes up more than 40% of the ash value. In exceptional cases, this percentage may not be reached. Lower potassium contents together with low percentages in the ash are a sign of inadmissible manipulations.

5.4 Sodium / Chloride

Grape juices made from grapes grown near the sea may have natural sodium contents of more than 30 mg/l. These products are not only characterized by an increased magnesium concentration; but, the chloride content which normally amounts to about 50 mg/l, will also be increased. A sodium content above 30 mg/l must correspond to the chloride value. It has to be taken into account that according to the "Zusatzstoff-Zulassungsverordnung"*, the addition of sodium sulphite, sodium hydrogen sulphite and sodium disulphite to grape juices is authorized. In practice, however, these materials are rarely ever used.*

5.5 Calcium

The calcium content of a grape juice is mainly influenced by the handling treatments applied. Freshly pressed grape juices which were not subjected to any further treatments, may have calcium values below 100 mg/l. Commercial juices seldom fail to meet this value. De-acidification with calcium carbonate which is sometimes applied, leads to increased calcium concentrations. The specified maximum value (350 mg/l) may be exceeded. De-acidified grape juices have a low tartaric acid content as compared to a high potassium concentration.

5.6 Magnesium

Unadulterated grape juices seldom have magnesium concentrations below 80 mg/l. If a juice remains far below this value it has to be analysed critically because together with other parameters, this low value may be a sign of water addition.

5.7 Phosphate

Normally, the natural phosphate content is lower than 500 mg/l; concentrations of more than 550 mg/l can only be expected in juices with high contents of extract and mineral substances.

5.8 Sulphate

In nature, grape juices contain only relatively small quantities of sulphate. The natural sulphate content is increased by sulphur dioxide which is used as a handling agent and/or for sulphurization (for provisional preservation) of the grape juice. "Desulphurized" grape juices may have increased sulphate contents. Depending on the storage period and/or the kind of desulphurization used, the guide value of 350 mg/l may be exceeded if the juices are stored for a longer period of time under sulphur dioxide in order to preserve them.

5.9 Nitrate

In nature, grape juices normally contain nitrate concentrations up to 15 mg/l. Due to a special plant physiology, these values may be exceeded by certain varieties.

6. Proline

The proline content is dependent on the degree of maturity as well as the fruit variety. It varies over a wide range. Natural proline values below the specified minimum value of 150 mg/l could only be observed in some white grape juices and may indicate the addition of water and/or the processing of rotten fruit.

7. Hydroxymethylfurfural (HMF)

The HMF-content is not a criterion by which can be judged whether a juice has been adulterated or not. If values of about 20 mg/l and more occur together with a cooked flavour, it may be supposed that there has been, a technologically avoidable, excess thermal stress acting in the course of production, storage or filling. In grape juices which have been carefully produced, HMF occurance is negligible. Juices from red grapes are more likely to form HMF than juices from white grapes. Depending on the influence of time and temperature, HMF can be formed during the storage of filled juices. In this case, however, a cooked flavour cannot be detected.

3.5 Grapefruit Juice

Grapefruit juice is made from fruit of Citrus paradisi. The RSK-values are based on extensive analyses of all varieties and provenances which are important for industrial processing (North, South and Central America, South Africa, Mediterranean region).

Comment

1. Relative Density 20°/20°C

Only in exceptional cases do grapefruit juices not come up to the minimum value laid down in the Leitsätze für Fruchtsäfte, i.e. 1.040. In these cases grapefruit juices with higher extract contents have to be used for blending. If the concentrate is reconstituted to single juice strength, the organoleptic and analytical characteristics must be equivalent to those of the original juice. The above-mentioned minimum value for relative density results from native fruit components.

Updated supplement to;

RSK-Values – The Complete Manual

1st Edition 1987

Lemon Juice

March 1993

(Please insert between page 38 and 39)

3. 11 Lemon Juice

Lemon juice is made from the fruits of Citrus limon. The RSK-values are based on extensive analyses of all varieties and provenances which are of significance to the fruit processing industry. In addition to cloudy juices, clear juices have also been included in the research. If the juices have been manufactured properly, the essential analytical parameters (except for hesperidin, pectin) show no deviation. Juices of Citrus aurantiifolia (also called lime or lime acid) were not considered.

Tab. 11: RSK-Values for Lemon Juice

All values referred to a relative density 20 °/20 °C of 1.035.

			guide value	range from	range to	central value
A. sensory analysis						
colour/appearance	(points)	min.	3	2	4	4
aroma	(points)	min.	3	3	6	4
flavour	(points)	min.	5	5	10	7
B. chemical analysis						
relative density 20 °/20 °C		min.	1.030	1.028	1.043	1.035
Brix, ref. corr.		min.	7.54	7.05	10.70	8.76
soluble solids	g/l	min.	77.7	72.5	111.6	90.7
titratable acids						
expr. as tartaric acid (pH 7.0)	g/l	max.	70	52	73	64
expr. as mval/l			933	693	973	853
expr. as citric acid (pH 8.1)	g/l	max.	61.5	46	64	56
expr. as mval/l			961	719	1000	875
L-malic acid	g/l	min.	1.4	1.0	7.5	–
citric acid	g/l	max.	60	45	63	55
D-isocitric acid	mg/l	min.	250	230	500	320
citric acid-isocitric acid ratio		max.	200	100	210	
tartaric acid	g/l			n.d.		
glucose	g/l		–	3	12	8
fructose	g/l		–	3	11	7.5
glucose-fructose ratio			–	0.85	1.3	1.1
sucrose	g/l		–	0	6.5	2
reduction free extract			–	65	82	72
ash	g/l		–	2.3	4.3	3.0
alkalinity number		min.	11	11	14.5	13
potassium (K)	mg/l	min.	1200	1100	1800	1400
in % in ash			–	42	50	46
potassium-magnesium ratio			–	13	22	17
sodium (Na)	mg/l	max.	30	–	–	–
magnesium (Mg)	mg/l		–	70	120	85
calcium (Ca)	mg/l	max.	130	45	160	85
chloride (Cl)	mg/l	max.	60	–	–	–
nitrate (NO_3)	mg/l	max.	10	–	–	–
phosphate (PO_4)	mg/l	min.	270	250	450	320
in % in ash			–	8	14	11
sulphate (SO_4)	mg/l	max.	100	–	–	–
formol number (ml 0.1 mol NaOH/100 ml)		min.	14	13	28	17
proline				100	800	
L-ascorbic acid	mg/l	min.	200	–	–	350

		guide value		range from	to	central value
flavonoid glycosides (acc. to Davis)						
expr. as Hesperidin	mg/l	max.	1200	400	1500	1000
water-soluble pectins expr. as galacturonic-acid anhydride	mg/l	max.	500	–	700	350

Tab. 11a: Additional Chemical Analysis of Lemon Juice

All values referred to a relative density 20 °/20 °C of 1.035.

	range from	to	central value
1. free amino acids, mmol/l			
aspartic acid	2.3	6.0	4.4
threonine	0.08	0.25	0.12
serine	1.3	3.5	2.4
asparagine	1.0	4.5	2.2
glutamic acid	1.1	2.7	1.90
glutamine	n.n.	0.3	–
proline	0.8	7.0	–
glycine	0.09	0.3	0.15
alanine	0.9	2.8	1.7
valine	0.07	0.3	0.17
methionine	traces	0.03	–
isoleucine	0.02	0.08	0.05
leucine	0.02	0.08	0.06
tyrosine	traces	0.04	–
phenylalanine	0.05	0.25	0.1
γ-aminobutric acid	0.6	1.8	1.1
ornithine	traces	0.04	–
lysine	0.03	0.15	0.07
histidine	traces	0.07	–
arginine	traces	0.6	0.3
2. ammonia	–	6.0	–
ethanolamine	–	0.5	–

Comment

1. Relative Density 20 °/20 °C

In order to obtain comparable values for direct juices and concentates, the analytical numbers have been referred to a relative density of 1.035. This corresponds approximately to the average value of all analysed direct juices and can serve as key value in the reconstitution of concentrates. Only few Argentinian and Italian juices remained somewhat below the lower range limit, whereas some Israelian juices slightly exceeded the upper range limit. The relative density is mainly determined by the citric acid content. The share of total sugar in the soluble solids amounts to approx. 20% on average.

2. Reduction free Extract

The amount of reduction free extract is mainly determined by the acid content of the juice. Especially South American juices tend to the upper part of the range, whereas juices from the Mediterranean region rather tend to the lower part. None of the juices fell below or exceeded the specified ranges.

3. Monosaccharides, Disaccharides

In most cases, lemon juices have a sucrose content below 2 g/l. A concentration of more than 6 g/l was not found. As a rule, the glucose content is higher than the fructose content. Glucose-fructose ratios below 1.00 are rare; values lower than 0.95 are a sign of microbiological degradation of the glucose. The upper value is hardly ever exceeded. Ratios above 1.2 must already be regarded as an exception. The highest glucose and fructose concentration can be detected in Israelian lemon juices. In a few cases, Israelian juices slightly exceeded the upper limit.

4. Fruit Acids

4.1 Titratable Acids

In the international concentrate trade, the content of titratable acid (pH 8.1 calculated as citric acid) is often expressed as GPL (grams per litre of concentrate). Juices from different countries of origin show no essential difference in the total acidity. If the reference value for relative density (20°/20°) is fixed at 1.035, juices from Argentina (South American provenance) tend to the upper part of the range.

4.2 Citric Acid

The citric acid content determines the share of titratable acid. For the quotient of titratable acid (pH 8.1 – calculated as citric acid) and citric acid, a central value of 1.02 and a variation between 0.95 and 1.10 were calculated.

4.3 D-Isocitric Acid

Mainly American and Israelian juices lie in the upper part of the range, whereas Italian juices are mostly in the lower part. In some cases, the specified maximum value is exceeded. A low content frequently occurs in juices with low acidity. A decrease of the D-isocitric acid content during the harvesting period can be noticed. Values below 230 mg/l were not observed.

4.4 Citric Acid-Isocitric Acid Ratio

The concentration of citric acid correlates with the isocitric acid concentration to some extent. Therefore, the ratio can be used to detect an acidification with citric acid. Ratios up to just under 100 were found in some cases. Only in a few authentic samples, the upper range limit was exceeded up to a quotient of 240. Setting down a central value is problematic since it depends

strongly on the fruit provenance, and therefore the central values calculated for the citric acid-isocitric acid ratio of South American, Californian, Spanish and Israelian juices are clearly below that of Italian lemon juices (approx. 180).

4.5 L-Malic Acid

The L-malic acid content depends on the provenance. For that reason, a central value cannot be specified. Juices of South American provenance are usually in the upper part of the range and their central value is higher than 4 g/l; juices from the Mediterranean region hardly exceed 4 g/l and have a central value of 2 g/l. Some Italian, Spanish and Israelian lemon juices may lie just below the lower range limit. Values below 1 g/l can also be the result of microbiological degradation.

5. Mineral Substances

5.1 Ash

The ash value is influenced, among other things, by the processing technique. The ash content shows no special features which might be due to provenance. A deviation from the specified range was not found. In the case of a high content together with a low potassium percentage in the ash, the juice has to be checked for a possible sulphurization treatment (cf. alkalinity number and sulphate).

5.2 Alkalinity Number

Juices whose alkalinity number is lower than 11 or higher than 14 should be checked critically for their anion and cation concentration respectively. Strong sulphurization may cause a decrease in the alkalinity number to less than 11.

5.3 Potassium

The potassium concentration correlates with the ash content. Between the juices from different countries of origin no essential difference in concentration has been observed. Values exceeding the upper range were not found. Only a few Italian juices remained slightly below the lower range limit.

5.4 Sodium

Values of more than 30 mg/l may indicate inappropriate processing or inadmissible treatments. Due to certain technological methods of processing used in the preceding stage of peel oil extraction, the sodium content may increase up to a maximum of 40 mg/l.

5.5 Calcium

The calcium content is strongly influenced by the fruit texture and the technology. Additions of pulp-wash and/or peel parts or extracts lead to an increase of the flavonoid glycosides

according to Davis (hesperidin) and water-soluble pectic substances as well as to an increase of the calcium concentration. The increased calcium content observed in juices from the Mediterranean region, which may even exceed the specified maximum value, must be considered a result of technology. Values below 50 mg/l calcium are extremely rare and must be regarded critically.

5.6 Magnesium

A dependence on the fruit variety and the fruit origin cannot be observed. Only in particular cases, the lower range limit of 70 mg/l may be slightly fallen below.

5.7 Phosphate

Values exceeding the upper range limit have not been observed. The minimum value may be slightly fallen below. There are no differences in the phosphate content which relate clearly to the fruit provenance; nevertheless, Italian juices generally tend towards lower values. The percentage of phosphate in the ash is largely independent of the fruit provenance as well; Italian juices again show somewhat lower values. A percentage of slightly more than 14 was found in a few South American juices, whereas values below 8 could not be detected.

5.8 Sulphate

Strongly sulphurized juices may exceed the guide value.

5.9 Nitrate

Lemons absorb virtually no nitrates; in lemon juice production, and as a result of the processing technique, nitrate adsorption is not possible. Lemon juices normally have a nitrate content of less than 5 mg/l. In juices made from concentrate, a nitrate content over 10 mg/l indicates that the water used does not have the appropriate characteristics.

5.10 Chloride

South American juices have a low chloride content, and values of more than 30 mg/l are extremely rare. In juices from the Mediterranean region the chloride content is usually higher than 30 mg/l and sometimes the guide value may be exceeded.

6. Formol number

Industrially processed juices showed almost no deviation from the range limits. The guide value is seldom fallen below.

6.1 Proline

The proline concentration depends strongly on the fruit provenance. The specification of a central value is not tenable. South American juices show the lowest proline values; in some

cases they are even below 100 mg/l. E.g. the central value of South American juices is about 200 mg/l, and values higher than 300 mg/l are rare, whereas for juices from the Mediterranean region, the central value is about 550 mg/l. Only in exceptional cases, the proline values were lower than 350 mg/l. Some juices from the Mediterranean region reached over 800 mg/l.

6.2 Amino Acids / Ammonia

Except for proline, the distribution of the individual amino acids is influenced neither by the fruit variety nor by the provenance. In Italian juices, a certain tendency to the lower range part can be observed for aspartic acid and γ-aminobutyric acid, whereas the glutamic acid concentration is in the upper range part. None of the juices fell below the minimum value for aspartic acid; values in the upper part and slightly above the upper range limit occur especially for lime juices. In some cases the upper range limit for serine, alanine and γ-aminobutyric acid may be slightly exceeded. In juices from overripe fruits, the content of γ-aminobutyric acid and ammonia lies in the upper range part. An increased arginine content indicates the use of juices from other citrus species.

7. Flavonoid Glycosides (calculated as Hesperidin) according to Davis

The content is strongly influenced by the fruit texture and the technology. Additions of pulp-wash and/or peel parts and extracts will lead to an increase. Whereas in South American juices the content is seldom higher than the guide value, the latter may be exceeded by juices from the Mediterranean region. The lower range limit is not fallen below. The "real" hesperidin value determined with high pressure liquid chromatography is lower than the "Davis-value" and varies between 250 and 900 mg/l. Naringin values over 10 mg/l indicate the use of parts of other citrus fruits (e.g. bergamot, grapefruit).

8. Pectic Substances

The content of pectic substances (water-soluble, oxalate-soluble, alkali-soluble) is influenced by the fruit texture and the technology. Increased values occur for water-soluble pectic substances, especially in Italian and Israelian juices. As a rule, the central value for juices from the Mediterranean region is higher than for juices coming from other countries of origin.

9. L-Ascorbic Acid

The content is strongly dependent on the fruit ripeness, the technology of juice production and especially the storage conditions. A content below 200 mg/l does not occur in properly manufactured and stored juices. Values over 500 mg/l are rare.

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